PECULIARITIES OF THE TERRESTRIAL LARVA OF THE URODELOUS BATRACHIAN, PLETHODON CINEREUS Green.

BY THOMAS H. MONTGOMERY, JR., PH.D.

To the writer's knowledge no description has been published of the larval stage of this strictly terrestrial species, which occurs through the United States of America east of the Mississippi river. Cope[1] states: "Its habits are entirely terrestrial, as it is never, even in the larval stage, found in the water. It is abundant under stones and logs in the forests everywhere, and does not occur in open fields. The eggs are laid in a little package beneath a stone in a damp place. When the young emerge they are provided with branchiæ, but these soon vanish, and they are often found in this young stage apparently quite developed." I have collected several hundred individuals near West Chester, Pa., at all seasons of the year, and have never found them in streams or boggy places, but most generally in woods on hillsides at varying elevations above water-courses, sometimes several hundred feet from any water, and occasionally in open fields and hillsides which in the summer season become very dry. For the most part they are found beneath wood and stones, and even in mid-winter may be found in these places, though at that time generally deeper in the ground than in summer.

This being, then, such a strictly terrestrial species, it was to be anticipated that its larval stage would show deviations from the larvæ of the other *Urodelea* which develop in the water. In July of the present year I found five eggs of *Plethodon cinereus* under a stone, and curled around them, on guard, an adult which dissection proved to be a female; these ova were larval stages, and the examination of them showed many interesting modifications, as follows:

The eggs are relatively very large for the size of the species, and each enclosed in gelatinous envelopes. Active movements of the heads and tails of the larvæ could be observed within the innermost membrane. But the striking peculiarity, even to the naked eye,

[1] "The Batrachia of North America," *Bull. U. S. Nat. Mus.*, No. 34, 1889.

is a large, nearly spherical yolk-mass around which the larva is curled, or rather into which it is pressed by the tension of the egg-envelopes. Plate XXX, fig. 1 shows a larva freed from its envelopes before killing so that it had straightened out; and fig. 2, an older larva, similarly treated, but which had still retained much of its normal position. The surface of the yolk-sphere is well supplied with blood-vessels, as shown in fig. 1, an antero-ventral one being particularly prominent. The figures show that there are three pairs of gills present (in fig. 2 only one gill is shown on the right hand, for the sake of clearness); and in the older larva the gills are of great size, much branched, the first the smallest, the second largest, lamellar and richly vasculated. The fore and hind limbs are already well marked, the toes on both faintly outlined; but most remarkable is the fact that the posterior limbs are larger than the anterior, which might indicate that the former develop first, in contradiction to what is known of other *Urodelea*. The head, the limbs, and all the trunk region of the embryos, except the end of the tail, are dorso-ventrally flattened, due undoubtedly to pressure against the yolk-sphere, but become more cylindrical after removal from the egg-envelopes. The head and trunk are pigmented with dark-brown chromatophores, which in the trunk region are arranged metamerically, while the yolk-sphere is not pigmented and of a yellow color; and as the figures show, the eyes are very large. A mouth was present in both cases, but there appeared to be no sucking disks upon the lower side of the head.

In fig. 3 is shown a camera drawing of a section through a stage somewhat younger than that of fig. 1; this section was made through an embryo curled closely around the yolk-sphere, in such a manner that the anterior region of the head, shown on the upper side, is cut medially, while a portion of the bend of the trunk, seen on the right-hand lower side, is cut obliquely. This figure is to illustrate the relations of the intestine to the yolk-mass. The mouth (*Mo.*) leads through the pharynx (*Ph.*) and œsophagus (*Œs.*) to the stomach (*St.*), and posteriorly to the latter is a short diverticulum (*D.*). The small intestine (*Int.*) is seen to be tubular in its proximal portion, but more distally to pass over into the wall of the yolk-mass (*Yk.*). The yolk-mass of this stage is seen to be composed of large yolk-cells, the boundaries of which are very distinct. In this fig. 3 the relative dimensions of

yolk-mass to the body are not shown, since the head hides a part of the yolk-mass, and since the head is cut obliquely and so appears larger than it would be in strictly median section.

But the relations of the intestine to the yolk-mass are more clearly shown in the camera drawings 4 and 5, which are dorsal portions of cross-sections of the larva shown in its entirety in fig. 1. Fig. 4 is in a plane behind the gill region, and shows how the intestine (*Int.*) is connected with the yolk-sphere (*Yk.*). The epithelium of the intestine, the nuclei of which are shown as black spots, expands ventrally into the yolk-sphere on the dorsal aspect of the latter. The epithelium of the intestine ends abruptly against the peripheral layer of the yolk-sphere, and does not pass over into it gradually. The nuclei of the yolk-sphere are much smaller and peripherally placed, and in places are found small clusters of nucleated cells. The yolk-mass of this larva shows that the cell boundaries of the yolk-cells have disappeared, and consequently the latter are undergoing regressive changes; the yolk appears as a mass of globules of different volumes suspended in a structureless fluid and without nuclei. Fig. 5 represents the dorsal half of a cross-section through the same larva, in a plane about half-way between the anterior and posterior limbs. In this region there is no tubular intestine, nor any open groove of intestinal epithelium upon the yolk-sphere, the entoderm being represented simply by the yolk-sphere (*Yk.*), the small nuclei of which are on its periphery.

These sections make clear the nature of the yolk-mass, and its relation to the intestine. From the mouth to the commencement of the small intestine the alimentary tract is tubular, and has the same appearance in its epithelial lining as in like stages of other *Batrachia;* the same is true also for the rectal region of the alimentary tract, which is likewise tubular. But the middle region of the intestine is composed of the yolk-mass, which in the earlier stage shown in fig. 3 is made up of large yolk-cells, and in the latter stage of figs. 4 and 5 of a mass of yolk-globules with a peripheral layer of small nucleated cells. Accordingly the large yolk-sphere is not a yolk-sac, since it is an integral part of the intestine.

The larva shown in fig. 1, of which figs. 4 and 5 represent sections, is in quite an advanced stage. Externally can be seen the

gills and both pairs of limbs. The sections show that pronephroi are present in the anterior trunk somites, and metanephroi (*M. T.*, fig. 5) in the posterior somites. There is a cartilaginous brain capsule, cartilaginous vertebral arches (*V. C.*, figs. 4 and 5), and cartilages for the bones of the limbs. The notochord (*N. C.*) is undergoing degeneration; the somatic mesoderm (*So. M.*) is divided into its various components—muscle somites, sclerotom, etc. The segmental ducts (*S. D.*) extend posteriorly and open into the rectum. The cœlom (*C.*) is large, the median mesenteries well formed (*M.*), the liver (*L.*) and hepatic ducts present. The epidermis (*Ect.*) is thick and glandular on the dorsal side of the body, and the genital ridges (*G. R.*, fig. 5) developed. All these points show an advanced stage of development, which makes it the more remarkable that the middle intestine should be represented by a large yolk-sphere.

The sides and ventral aspect of the yolk-sphere are covered with a very thin envelope composed of three cell layers closely apposed, the flattened ectoderm (*Ect.*, figs. 4, 5), the somatic mesoderm (*So. M.*) and the splanchnic mesoderm (*Sp. M.*). The fact that the body wall is excessively thin upon the ventral side of the yolk-sphere, would show perhaps that this wall had extended around the yolk not until late in the development. The blood-vessels of the yolk-sphere have their position in the mesodermal layers.

In conclusion, we find the principal modification of these larval stages to consist in the presence of a large yolk-sphere, which is an integral part of the mid-gut, while the anterior and posterior portions of the gut are tubular. Peculiar also is the great size of the posterior limbs and of the gills (fig. 2), and the continuance within the egg-envelopes after a time when in other *Urodelea* the larva has emerged from them. All these modifications must be referred to the terrestrial life; and the great size and long continuance of the yolk-sphere may be accredited the value of a source of nourishment. A life under still dryer surroundings, and longer life within the egg-envelopes necessitating a larger yolk-sphere, might lead to the formation of a yolk-sac in the strict sense by the holoblastic cleavage becoming mesoblastic. The case of *Plethodon cinereus* is but another to show how readily developmental processes become modified by change of the environment, and how much care must be used in interpreting them in the search for affinities.

A number of cases are known in the *Anura* of terrestrial larvæ with a large yolk-sphere, which have been collected together in a contribution by Miss L. V. Sampson,[2] but terrestrial development in the *Urodelea* appears to be much less frequent. In the Cæcilian genus, *Ichthyophis*, the embryology of which has been carefully studied by the Sarasins,[3] there is a large yolk-sphere, which at first becomes segmented only peripherally and not until much later through its entire mass, so that here the development is at first mesoblastic much as in a Sauropsidan. That may perhaps be found to be the case in *Plethodon* also when its early cleavage is studied. In *Ichthyophis* the intestine lies at first as a straight open groove upon the yolk (cf. the chapter in the Sarasins' monograph, *Das Schicksal des Dotters*) ; then the yolk bends into a number of lobes, which later become elongated and entirely covered by the body wall. The intestinal groove of *Ichthyophis* is supposed by these investigators to become a closed tube without growing round the yolk-mass, but they did not have the necessary stages to show the final fate of the yolk. The Sarasins laid particular stress upon the peculiar development of the *Cæcilia* in discussing their relationships, as, *e.g.*, in allying them with *Amphiuma*, which Hay[4] has shown to have quite a similar development. But the fact that *Amphiuma*, the *Cæcilia* and *Plethodon* show great similarity in their development, might prove rather that the formation of a large yolk-mass with the embryo curled around it may be merely the consequence of terrestrial development, and the similarity express rather a case of convergence than of phyletic affinity. The relationships of the *Amphibia* must be shown from comparative anatomical standpoints, and not from the larval development which obviously may be easily modified by change in environment, as is particularly well shown in the *Anura*. Brauer, who studied the development of the Cæcilian genus *Hypogeophis* from the Seychelles, where it lives wholly terrestrial, concludes:[5] "Wenn auch kein Zweifel darüber aufkommen kann,

[2] "Unusual Modes of Breeding and Development Among Anura," *American Naturalist*, 34, 1900.

[3] "Ergebnisse naturwissenschaftlicher Forschungen auf Ceylon," Wiesbaden, 1887–1890.

[4] "Observations on Amphiuma and Its Young," *American Naturalist*, 1888.

[5] "Beiträge zur Kenntniss der Entwicklungsgeschichte und der Anatomie der Gymnophionen," *Zool. Jahrb.*, 10, 1897.

dass die grosse Dottermasse bei den untersuchten Gymnophionen erst secundär erworben ist, nachdem die Entwicklung nicht mehr im Wasser ablief, sondern ganz auf dem Lande, ... so ist deshalb auch die Annahme, dass die Entwicklung durch die grössere Dottermasse derart modificirt sei, dass ein Vergleich mit den übrigen Amphibien nicht berichtigt sei, meiner Ansicht nach nicht zutreffend. . . . . Aus diesen Betrachtungen ergiebt sich somit, dass der Keim der Cöcilien im Wesentlichen denselben Bau am Ende der Furchung hat wie der übrigen Amphibien und dass die scheinbar meroblastische Furchung in Wirklichkeit nur eine durch den grössern Dottergehalt bedingte Variation der inäqualen Furchung anderer Amphibien ist.''

The only other case known to me of a Urodele with terrestrial development (except the European viviparous *Salamandra maculosa*) is that of the Californian *Autodax lugubris*, as described by Ritter and Miller.[6] In this species the eggs are laid attached by pedicels to stones on the land, there is a large yolk-sphere, large gills, and apparently quite close similarity to the larval stages of *Plethodon*.

[6] ''A Contribution to the Life History of *Autodax lugubris* Hallow.,'' *American Naturalist*, 33, 1899.